Colony PCR

Material	Volume	Concentration
dH20	67 QS 100µL	-
ThermoPol Buffer	5 µL	-
dNTPs (50x)	1 µL	10 mM
Fwd Primer (20x)	2.5 µL	10 µM
Rev Primer (20x)	2.5 µL	10 µM
VentR DNA Polymerase (100x)	1 µL	100x stock

1. Suspend colonies in 20µL of dH2O using toothpick
2. Transfer toothpick to 3mL LB + Ab
3. Add 1µL of cell suspension to 9µL master mix
4. Thermal Cycling:

Temp. (°C)	Time	Cycle
95	0:30	
95	0:30	
*	0:30	35X
68	0:30†	
68	5:00	
4	∞	

*Lowest primer annealing temperature
†30sec per 1kb

5. Grow correct length colonies overnight
6. Spot 3µL onto master plate (for later recovery)
7. Miniprep remaining culture and send for sequencing
8. After successful sequencing:

Day 1:

1. Start 2 overnight liquid culture (16-18 hours) of correct colony from master plate